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Short communication

Serologic responses of cats against experimental *Sarcocystis neurona* infections

J.P. Dubey^{a,*}, D.S. Lindsay^b, W.J.A. Saville^c

^a United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Parasite Biology, Epidemiology and Systematics Laboratory, Building 1001, Beltsville, MD 20705-2350, USA

^b Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Disease, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech., 1410 Prices Fork Road, Blacksburg, VA 40061-1092, USA

^c Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210-1092, USA

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Abstract

Sarcocystis neurona is the most important cause of a neurologic disease of horses, equine protozoal myeloencephalitis (EPM). Cats and other carnivores can act as its intermediate hosts and horses are aberrant hosts. Little is known of the sero-epidemiology of *S. neurona* infections in cats. In the present study, antibodies to *S. neurona* were evaluated by the *S. neurona* agglutination test (SAT). Cats fed sporocysts from the feces of naturally infected opossums or inoculated intramuscularly with *S. neurona* merozoites developed high levels ($\geq 1:4000$) of SAT antibodies. Antibodies to *S. neurona* were not found in a cat inoculated with merozoites of the closely related parasite, *Sarcocystis falcatula*. These results should be useful in studying sero-epidemiology of *S. neurona* infections in cats. Published by Elsevier Science B.V.

Keywords: *Sarcocystis neurona*; Agglutination test; Antibodies; *Sarcocystis falcatula*; Cats

1. Introduction

Sarcocystis neurona is the most important cause of a neurological disease of the horse (equine protozoal myeloencephalitis—EPM) and EPM is the most commonly diagnosed neurologic disease of the horse in the US (Dubey et al., 2001b). It has an unusual life cycle. Cats, raccoons, armadillos, skunks, and sea otters can act as its intermediate hosts and

* Corresponding author. Tel.: +1-301-504-8128; fax: +1-301-504-9222.

E-mail address: jdubey@anri.barc.usda.gov (J.P. Dubey).

opossums are its definitive hosts (Dubey et al., 2000, 2001b,c,d; Cheadle et al., 2001a,b; Tanhauser et al., 2001). Opossums excrete sporocysts in their feces after ingesting tissues of intermediate hosts that harbor the sarcocyst. Intermediate hosts become infected by ingesting food and water contaminated with sporocysts. Horses are its aberrant host and become infected by ingesting sporocysts (Fenger et al., 1997; Dubey and Lindsay, 1998). However, unlike intermediate hosts, sarcocysts are not formed in the horse; only schizonts are found in tissues of the horse (Dubey et al., 1991).

Little is known of the sero-epidemiology of *S. neurona* in the intermediate hosts. Recently, antibodies to *S. neurona* were found in 58.6% of 99 raccoons (Lindsay et al., 2001). The objective of the present study was to investigate antibody responses of cats experimentally infected with *S. neurona*.

2. Materials and methods

2.1. Infections of cats

Parasite-free cats from a closed colony were used. The management and history of these cats has been reported (Dubey, 1995). Three experiments were performed.

In experiment 1, cats had been fed sporocysts of the SN 15-OP isolate of *S. neurona* from a naturally infected opossum (Dubey et al., 2000). These cats were the same as used for studying the life cycle of *S. neurona* (Dubey et al., 2000). The cats were bled and killed on days indicated in Table 1.

In experiment 2, four 61–85-day-old cats were inoculated subcutaneously (sc) with 10⁶ or more culture-derived merozoites of *S. neurona* (cats 542, 544, 552) or a *Sarcocystis falcatula*-like parasite (cat 543) (Table 2). The *S. neurona* isolates were obtained in cell cultures inoculated with brains of interferon gamma gene knockout (KO) mice that had been fed sporocysts from naturally infected opossums as described (Dubey, 2000; Dubey et al., 2001a). The *S. falcatula* isolate was obtained in cell culture seeded with lung tissue of a budgerigar (*Melopsitticus undulatus*) No. 152b that had been fed sporocysts from a naturally infected South American opossum (*Didelphis marsupialis*) from Brazil (Dubey et al., 2001b). The cats were bled and killed as indicated in Table 2.

Table 1
S. neurona agglutination antibodies in cats fed sporocysts from a naturally infected opossum

| Cat no. | Cortisone given | Day killed | Antibody titers day post-inoculation | | | | | |
|------------------|-----------------|------------|--------------------------------------|-----|-------|-------|-----------------|---------|
| | | | 0 | 7 | 20 | 35 | 49 | 144–167 |
| 511 | Yes† | 36 | <25 | 25 | 40000 | 40000 | ND ^a | |
| 525 | Yes† | 144 | <25 | 25 | 4000 | 40000 | 20000 | 40000 |
| 536 | No | 144 | <25 | <25 | 800 | 800 | 800 | 800 |
| 495 ^b | Yes | 167 | <25 | <25 | 40000 | 40000 | 16000 | 40000 |
| 499 ^b | Yes | 167 | <25 | <25 | 16000 | 16000 | 64000 | 64000 |

^a Not done.

^b Muscles from these cats were fed to two cats (No. 573, 574) and two dogs (No. 28, 29).

Table 2

Sarcocystosis in cats inoculated sc with merozoites of *S. neurona* or *S. falcatula*

| Cat no. | Organism | Dose | Day killed | Bioassay in opossum no. | Antibody titers at weeks post-inoculation | | | | | |
|---------|---------------------|--------------------|------------|--------------------------|---|-----|------|------|-------|-------|
| | | | | | 0 | 2–3 | 4–5 | 7–8 | 8–9 | 14 |
| 543 | <i>S. falcatula</i> | 4.6×10^6 | 101 | 1 Negative 2 Negative | <25 | 25 | <25 | <25 | <25 | <25 |
| 552 | <i>S. neurona</i> | 1.25×10^7 | 105 | 3 Negative 4 Positive | <25 | <25 | 25 | 25 | 25 | 6400 |
| 544 | <i>S. neurona</i> | 1×10^7 | 105 | 5 Negative | <25 | 500 | 5000 | 5000 | 64000 | 64000 |
| 542 | <i>S. neurona</i> | 2×10^6 | 103 | 6 Negative 7 Negative | <25 | 100 | 100 | 500 | 500 | 25 |

In experiment 3, six 72–76-day-old cats were fed $\sim 10^6$ sporocysts of the isolate SN 30-OP; the sporocysts had been stored at 4 °C for 9 months (Dubey et al., 2001a). Four cats were given methyl prednisolone acetate intramuscularly 7 days before and 14 and 40 days after feeding sporocysts (DAFSs) (Table 3). The cats were bled and killed on the days indicated in Table 3. Skeletal muscles from four cats (two given cortisone and two not given cortisone) were fed to laboratory-raised opossum Nos. 13, 22, 23 and 24. The opossums were raised as described by Dubey et al. (2000).

2.2. Bioassay of cat muscles in opossums, dogs and cats

Muscles of certain cats were fed to laboratory-raised opossums (Tables 2 and 3) as described (Dubey et al., 2000). In addition to opossums, muscles from cats 495 and 499 were pooled and fed to two cats and two laboratory-raised dogs (Table 1). Feces of opossums, dogs, and cats were examined for sporocysts. If no sporocysts were found in feces, small intestinal homogenate was digested in 5.25% sodium hypochlorite (Clorox) and the digest examined for sporocysts as described (Dubey et al., 2001d). The number of sporocysts shed were counted in intestinal homogenates of opossums as described (Dubey, 2000).

Table 3

S. neurona infection in cats fed sporocysts of the SN 30-OP isolate

| Cat no. | Cortisone, mg/kg (total mg) ^a | Day killed | Fed to opossum no. | No. of sporocysts shed | Antibody titers days post-inoculation | | | | |
|---------|--|------------|--------------------|------------------------|---------------------------------------|-------|-------|-------|---------------------|
| | | | | | –7 | 14 | 42 | 74 | 82–178 ^b |
| 598 | 5 mg (20) | 142 | 22 | 6×10^6 | <50 | 64000 | 64000 | 64000 | 4000 |
| 599 | 5 mg (20) | 178 | No | NA | <50 | ND | 64000 | 64000 | 64000 |
| 600 | 10 mg (35) | 82 | 13 | 29×10^6 | <50 | 1000 | 64000 | 64000 | 16000 |
| 601 | 10 mg (40) | 178 | No | NA | <50 | 10 | 64000 | 64000 | 64000 |
| 602 | None | 144 | 24 | $<10^6$ | <50 | 1000 | 100 | 100 | 100 |
| 603 | None | 144 | 23 | 1.2×10^6 | <50 | 100 | 64000 | 64000 | 4000 |

^a Methylprednisolone acetate was injected intramuscularly on days –7, 14 and 40.

^b Day 82 (cat 600), day 144 (cats 598, 602, 603), day 178 (cats 599, 601).

2.3. *S. neurona* agglutination test (SAT)

The SAT as described by Lindsay and Dubey (2001) was used to determine antibodies in sera of cats.

3. Results

Antibodies to *S. neurona* were found in sera of cats by 21 DAFS, whether cats were given cortisone or not. However, antibody titers in cats given cortisone were higher than those not given cortisone (Tables 1 and 3). The cat inoculated with *S. falcatula* merozoites remained seronegative ($<1:25$) (Table 2), except for a low titer of 1:25 on day 28 (Table 2). All three cats inoculated with *S. neurona* merozoites developed antibodies to *S. neurona* (Table 2).

Opossums fed muscles of cats that had been fed sporocysts shed few to 29 million sporocysts (Table 3). The two cats and two dogs fed *S. neurona* sarcocysts did not shed sporocysts.

4. Discussion

Results of this investigation demonstrate that cats fed *S. neurona* sporocysts can develop high levels of antibodies measured in the SAT. All cats had no detectable antibodies to *S. neurona* before the start of the experiment. The cats used were parasite-free. The study was not designed to investigate sensitivity and specificity of SAT in cats, although one cat (No. 543) inoculated with merozoites of a closely related parasite *S. falcatula* did not develop antibodies to *S. neurona*. We did not have access to sera from cats infected with other species of *Sarcocystis* (Dubey et al., 1989) or from cats with natural *S. neurona* infection (Turay et al., 2002).

In the present study one cat (No. 552) inoculated with *S. neurona* merozoites developed *S. neurona* sarcocysts that were infective to opossums. Thus, it was possible to obtain sporocysts derived from *S. neurona* merozoites. This finding is of interest because until now it has not been possible to obtain sporocysts from horse-derived isolates of *S. neurona*. In addition, *S. neurona* has not been demonstrated in tissues of horses fed *S. neurona* sporocysts (Dubey et al., 2001b).

S. neurona has an unusual life cycle with a wide range of intermediate hosts and opossums as the only definitive host. In the present study, dogs and cats fed *S. neurona* sarcocysts did not shed sporocysts suggesting that they are not definitive hosts for *S. neurona*.

Shedding of *S. neurona* sporocysts in the present study confirms our earlier findings of cats as being an intermediate host for *S. neurona* (Dubey et al., 2000). A recent report indicates that cats in nature are also an intermediate host for *S. neurona* (Turay et al., 2002). Although feces of laboratory-raised opossums were not monitored before feeding infected tissues, there is no likelihood at all that they could be infected naturally with *Sarcocystis*.

Antibodies to *S. neurona* (SAT titers $\geq 1:25$) were found in 13% of 310 cats from rural Ohio (J.F. Stanek, R.W. Stich, J.P. Dubey et al., 2002). Antibodies to *S. neurona* were not found in 1:25 dilution of serum from 502 domestic cats from the city of São Paulo, Brazil, providing suggestive evidence for the specificity of the SAT; 26.3% of these cats had antibodies to *T. gondii* in modified agglutination test (Dubey et al., 2002).

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